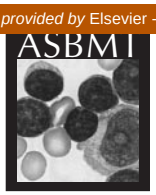
Biology of Blood and Marrow Transplantation 8:268-272 (2002)



Effect of Prior Therapy and Bone Marrow Metastases on Progenitor Cell Content of Blood Stem Cell Harvests in Breast Cancer Patients

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Received March 1, 2001; accepted February 20, 2002

ABSTRACT

This study was designed to examine the relationship of prior therapy, bone marrow metastases, mobilization, and blood progenitor/stem cell (BSC) collection in breast cancer patients. Cells were collected from 19 breast cancer patients during steady state (nonmobilized group) and from 69 breast cancer patients after cytokine administration (mobilized group). Characteristics of the patients were compared with the cells obtained. A significant inverse association was found between the number of chemotherapy regimens the patients had received prior to BSC collection and the mononuclear cell (MNC) count of the product per liter of blood processed (LBP) with apheresis (P = .0006) and the granulocyte monocyte/macrophage colony-forming cell (GM-CFC) numbers per LBP (P = .0002). This association was evident in both mobilized and nonmobilized patients. Similar results were seen in those 25 patients who had received prior radiation therapy (MNC/LBP, P = .0003; GM-CFC/LBP, P = .0004). Patients in both the mobilized and nonmobilized groups with marrow metastases at the time of collection also had significantly lower levels of MNC/LBP (P = .0039) and GM-CFC/LBP (P = .0001) than did those without marrow metastases. The findings suggest that prior administration of radiation therapy and/or chemotherapy and the presence of marrow metastases all negatively impacted the collection of mobilized and nonmobilized progenitor cells from breast cancer patients. The mechanisms of this impact are not understood.

KEY WORDS

Blood progenitor cell collection • Breast cancer • Bone marrow metastases

INTRODUCTION

Although autologous blood progenitor/stem cell (BSC) transplantation has been used in the treatment of breast cancer, limited information is available regarding factors influencing collection of adequate numbers of stem and progenitor cells from these patients. Prior treatment with radiation and chemotherapy is known to be associated with lower numbers of progenitors in mobilized autologous BSC collections [1,2]. Mice that have received radiation to various parts of their bodies exhibit markedly inhibited mobilization regardless of the area irradiated [3]. Previous radiotherapy and chemotherapy and an older age have had adverse effects on the efficiency of collection of mobilized $CD34^+$ cells, whereas the type of underlying malignancy and bone marrow infiltration by malignant cells have not had adverse effects [4].

Steady-state (ie, nonmobilized) blood stem cell collections were performed at some centers prior to 1991 [5]. Subsequently, hematopoietic cytokines (granulocyte colony-stimulating factor or granulocyte-macrophage colony-stimulating factor), sometimes in association with myelotoxic chemotherapy, have been employed to produce mobilization. Differences between collection characteristics of mobilized and nonmobilized cells and their relationship to bone marrow metastasis of autologous donors are unknown. The purpose of this study was to examine the relationship of prior therapy, mobilization, marrow metastases, and BSC collection among women with breast

cancer preparing for high-dose therapy and autologous BSC transplantation.

MATERIALS AND METHODS

Patients and Collections

Blood stem cells were collected from 88 stage II to IV breast cancer patients between 1987 and 1998. Eighteen of these patients had cells collected prior to April 1991 during steady state (nonmobilized group). The remainder had cells collected following cytokine administration (mobilized group). Histopathological analysis of bilateral marrow core biopsy specimens and aspirates was performed prior to cell collection to determine the adequacy of hematopoiesis and to detect the presence of tumor. The following data were determined retrospectively from medical records: the total volume of blood processed by the apheresis machine to collect the product, the total mononuclear cell (MNC) count per kilogram of body weight in the graft product and per liter of blood processed (LBP), the total number of granulocyte-macrophage colony-forming cells (GM-CFC) per kilogram of body weight in the graft product and per LBP, whether or not prior radiotherapy had been given, the number of prior chemotherapy regimens received (a regimen was defined as a combination of specific cytotoxic agents administered in regular cycles and could include adjuvant regimens such as 5 fluorouracil, methotrexate, and cyclophosphamide), and the number of months myelotoxic chemotherapy had been given prior to collection. This study was based on those 88 consecutive patients with complete MNC and GM-CFC data whose marrow biopsy specimens had been evaluated for marrow metastases using the immunohistochemical, immunocytochemical, and/or histopathologic methods described below. Because $CD34^+$ cell counts were available only for the mobilized group, the analysis was focused on MNC counts and GM-CFC numbers. Data are presented separately for all patients and for patients undergoing cytokine mobilization.

Apheresis Procedures

Sequential mobilization regimens employed daily administration of granulocyte-macrophage colony-stimulating factor (GM-CSF) at 125 $\mu g/m^2$ and then at 250 $\mu g/m^2$, followed later by administration of G-CSF 10 $\mu g/kg$ subcutaneously, with collections using the Cobe Spectra (Gambro BCT, Lakewood, CO) beginning on the fifth day of cytokine administration. Multiple apheresis procedures to collect a target MNC dose of 6.5×10^8/kg were required for the nonmobilized group. The target for mobilized autografts was also 6.5×10^8 MNC/kg plus 2.5×10^4 GM-CFC up to 1994 and subsequently at least 1.1×10^6 $CD34^+$ cells/kg. All patients participated in Institutional Review Board–approved stem cell collection studies, and each patient provided written informed consent for the collections and the analysis of their collections.

Hematopoietic Colony Assays

The numbers of GM-CFC in the collections were determined using a previously described culture technique [6]. Briefly, 3×10^5 MNCs that had been separated from the harvest were plated in 35-mm Petri dishes containing Iscove's modified Dulbecco's medium, 20% fetal bovine serum, 50 μM 2-mercaptoethanol, 100 $\mu g/mL$ streptomycin, 100 U/mL penicillin, and 0.3% agar supplemented with 200 U/mL interleukin-3, 200 U/mL GM-CSF, and 200 U/mL G-CSF. Cultures were incubated at 37°C in a fully humidified atmosphere of 5% CO_2 in air for 14 days. Aggregates greater than 50 cells were counted as colonies.

Bone Marrow Metastases and Micrometastases

Bilateral core biopsy specimens and aspirates obtained just prior to cell collection were examined histopathologically for the presence of tumor cells. In addition, sections of cores were stained immunohistochemically for cytokeratins using the MAK 6 antibody cocktail (Zymed, South San Francisco, CA). Patients with morphological or immunohistochemical evidence of tumor cells in their marrow were characterized as histologically positive for bone marrow metastasis. In addition, cytospin preparations were prepared from marrow aspirates and stained immunocytochemically for cytokeratins using the MAK 6 and Cam 5.2 (Becton Dickinson, San Jose, CA) antibodies as described previously [7]. Cells containing these cytokeratins were identified by indirectly linking the anticytokeratin antibodies to glucose oxidase, which was used as the indicator enzyme. Nitroblue tetrazolium, which produces a purple-blue precipitate color when reduced, was used as the substrate. Criteria for a positive result included immunoreactivity of at least 70% in the cytoplasm and morphology consistent with that of tumor cells. The characteristic cytoplasmic membrane staining was observed using a light microscope. Patients with histopathologic, immunohistochemical, or immunocytochemical evidence of tumor-positive cells in bone marrow were designated as bone marrow positive.

Data Analysis

The Kruskal-Wallis test was used to test the relationship between product characteristics and prior chemotherapy. The Wilcoxon test was used to test the relationship between product characteristics and prior radiation therapy, mobilization, and the presence of marrow metastases. Fisher's exact test was used to test the relationship between prior chemotherapy and the presence of marrow metastases.

RESULTS

Patient Characteristics

The median age of the patients in this study was 42 years (range, 30-62 years). Fifty-one (58%) of the 88 patients had received 1 prior chemotherapy regimen; 28 (32%) had received 2 prior regimens; 8 (9%) had received 3 prior regimens; and 1 patient had received 5 prior regimens. The median number of months these patients had received cytotoxic chemotherapy was 5 (range, 2-27 months). Twenty-five patients (28%) had received prior radiation therapy. Twenty-three (26%) of the 88 patients had immunocytochemically detectable tumor cells in their bone marrow at the time of blood stem cell collection.

Association of Prior Treatment and Collection Characteristics

No significant association was found between the number of prior chemotherapy regimens and the MNC count/kg

Table 1. *Cell Composition of Graft Products and Number of Prior Chemotherapy Regimens Received*

No. of Prior Regimens	No. of Patients	No. of MNC × 10^8/LBP, Median (Range)	No. of GM-CFC × 10^4/kg, Median (Range)	No. of GM-CFC × 10^4/LBP, Median (Range)
1				
All	51	13.2 (2.8-25.1)	10.4 (0.4-370.0)	14.0 (0.6-611.7)
Mobilized only	45	14.2 (2.8-25.1)	13.8 (2.2-370.0)	28.4 (1.0-611.7)
2				
All	28	9.6 (4.1-15.0)	3.8 (0.2-32.8)	2.5 (0.1-13.6)
Mobilized only	19	10.8 (4.1-15.0)	5.3 (1.1-32.8)	6.0 (0.5-66.0)
>2				
All	9	8.9 (4.1-12.7)	2.5 (0.1-13.6)	2.2 (0.1-16.5)
Mobilized only	5	9.8 (4.1-12.7)	3.1 (2.5-13.6)	3.5 (2.2-16.5)
Significance levels				
All		$P = .0006$	$P = .0003$	$P = .0002$
Mobilized only		$P = .022$	$P < .01$	$P = .015$

patient wt in the graft product ($P = .36$), but a significant inverse association between the number of prior chemotherapy regimens and MNC count/LBP ($P = .0006$) was observed in all patients and in cytokine-mobilized patients. A significant inverse association was also found for both the number of GM-CFC progenitors/kg ($P = .0003$) and GM-CFC numbers per LBP ($P = .0002$) in both groups. A dose-response relationship was apparent, with more heavily pretreated patients having lower counts (Table 1). Similar results were found for patients with prior radiation therapy (Table 2). The equivalence of these results for all patients, as well as the mobilized patient cohort alone, demonstrated that these effects were not a consequence of the mobilizing regimen.

Association of Mobilization and Collection Characteristics

The mobilized group had significantly higher levels of MNC/kg ($P = .0046$), MNC/LBP ($P = .0007$), GM-CFC/kg ($P = .0001$), and GM-CFC/LBP ($P = .0001$). The increase in GM-CFC numbers was approximately 10-fold, and more modest increases in MNC counts were found (data not shown).

Association of Marrow Metastases and Collection Characteristics

All patients whose bone marrow contained detectable tumor cells at the time of blood stem cell collection had collections that contained significantly lower levels of MNC, with a median of 7.2×10^8/kg (range, $6.0\text{-}23.0 \times 10^8$/kg) versus 9.2×10^8/kg (range, $3.4\text{-}25.7 \times 10^8$/kg) for 65 marrow-negative patients ($P = .0063$). Similarly, bone marrow–positive patients compared to bone marrow–negative patients had a median MNC of 8.9×10^8/LBP (range, $4.1\text{-}18.4 \times 10^8$/LBP) versus 11.5×10^8/LBP (range, $3.8\text{-}25.1 \times 10^8$/LBP) ($P = .0039$), GM-CFC of 1.1×10^4/kg (range, $0.1\text{-}58.1 \times 10^4$/kg) versus 8.4×10^4/kg (range, $0.1\text{-}370.0 \times 10^4$/kg) ($P = .0001$), and GM-CFC of 1.1×10^4/LBP (range, $0.1\text{-}93.5 \times 10^4$/LBP) versus 10.1×10^4/LBP (range, $0.3\text{-}611.7 \times 10^4$/LBP) ($P = .0001$). Although the number of GM-CFC expressed both as 10^4/kg body wt and 10^4/LBP was significantly higher for the mobilized than for the nonmobilized group, bone marrow tumor cell positivity was associated with lower MNC counts ($P = .029$) and GM-CFC levels in the collections of both groups, indicating that marrow positivity decreased collection efficiency, independent of cytokine administration.

Association of Marrow Metastases and Prior Chemotherapy

The relationship of prior chemotherapy and the detection of tumor cells in the marrow was examined separately for the mobilized and nonmobilized groups. No significant relationship was found in the nonmobilized group between the number of prior chemotherapy regimens and bone marrow positivity. The relationship between the number of prior chemotherapy regimens and bone marrow metastases

Table 2. *Cell Composition of Graft Products and Prior Radiation Therapy*

Prior Radiation	No. of Patients	No. of MNC × 10^8/LBP, Median (Range)	No. of GM-CFC × 10^4/kg, Median (Range)	No. of GM-CFC × 10^4/LBP, Median (Range)
Yes				
All	25	8.4 (4.1-15.2)	3.0 (0.1-14.5)	2.5 (0.1-25.8)
Mobilized only	17	8.7 (4.1-15.2)	5.0 (1.1-14.6)	4.8 (0.5-25.8)
No				
All	63	11.5 (3.8-25.1)	8.5 (0.1-370.0)	10.1 (0.2-611.7)
Mobilized only	52	12.7 (3.8-75.1)	11.5 (2.2-370.0)	13.6 (1.1-611.7)
Significance levels				
All		$P = .0003$	$P = .0013$	$P = .0004$
Mobilized only		$P < .01$	$P \leq .01$	$P < .001$

in the mobilized group was suggestive of an increased tumor cell positivity in the marrow in patients receiving more courses of chemotherapy, but this effect did not attain statistical significance ($P = .069$).

DISCUSSION

In this retrospective analysis, a statistically significant inverse association was found between the number of prior chemotherapy regimens patients had received, prior administration of radiation therapy, and the presence of bone marrow metastasis and the numbers of MNC/LBP and GM-CFC/LBP harvested from both breast cancer patients undergoing cytokine-mobilized and those undergoing nonmobilized blood stem cell collection. The negative effect of prior chemotherapy and radiation therapy on blood stem cell collections from breast cancer patients has been noted previously [8]. Also, in patients with non-Hodgkin's and Hodgkin's lymphoma, Haas et al. [9] noted that prior radiation therapy inhibited stem/progenitor cell collection. The assumption was that these prior therapies had damaged the stem cell compartment, thereby reducing stem cell numbers and/or preventing mobilization of large numbers to the circulation in response to cytokines, although other mechanisms might also have been operative. The sites receiving radiation therapy were not determined, but for this group of patients, it is likely that the chest wall and axilla predominated. Consequently, it is unlikely that the iliac crest bone marrow biopsy sites of these patients were irradiated. Concurrent administration of radiation and mobilizing cytokines to mice inhibits mobilization [3]. Because the effect was independent of the body area irradiated, a circulating factor was suspected to be involved. Injection of plasma from part-body irradiated mice into naive mice prior to injecting mobilizing cytokines also inhibited mobilization [3]. Preliminary studies have suggested this plasma from part-body irradiated mice contained elevated levels of transforming growth factor-β (TGF-β) [10].

Plasma from poorly mobilizing patients and from healthy donors of blood stem cells for allogeneic transplantation also inhibited mobilization when injected into naive mice prior to mobilization attempts [11]. Therefore, along with stem cell damage, patients who received prior radiation therapy and/or chemotherapy may have a circulating inhibitor of stem cell mobilization, which could decrease the efficiency of stem cell collection both in nonmobilized and in cytokine-mobilized patients as observed in this study. The nature of this inhibitor is uncertain, but breast cancer patients have been shown to have higher systemic levels of TGF-β that are similar to levels found in part-body irradiated mice [12]. Whether these elevated levels of TGF-β have a relationship to the extent and duration of prior therapy remains to be determined.

Some studies [4], but not all [13], have not found an association between poor BSC collection and marrow metastases. The current study suggested that collection of progenitors was less successful if marrow metastases were detected. The apparent discrepancies in reported studies might reflect the sensitivity of the methods used to detect marrow metastases. This study used relatively sensitive immunocytochemical and immunohistochemical techniques as well as standard histopathologic methods.

These observations suggest that an evaluation of the prior therapy history of the patients and the metastatic or micrometastatic status of their bone marrow should identify those breast cancer patients more likely to require a greater number of aphereses or an increased apheresis volume to achieve an adequate BSC collection. This study also emphasizes that the mechanisms responsible for poor mobilization are not entirely understood.

ACKNOWLEDGMENTS

A.D. was a recipient of a National Institutes of Health fellowship. This support is gratefully acknowledged.

It is a pleasure to thank Mrs. S. Mann, Dr. Barbara O'Kane Murphy, and J. Cannella for excellent technical assistance; the transplant coordinators; pathologists; apheresis nurses; and Penni Davis, who typed the manuscript.

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